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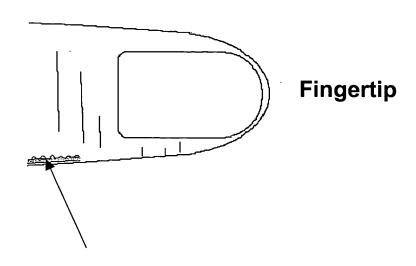
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Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000 Filed: HEREWITH

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Reporter and Marker colors

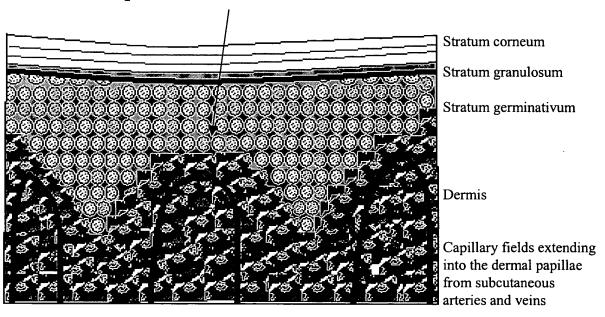
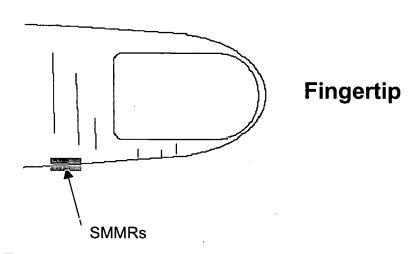


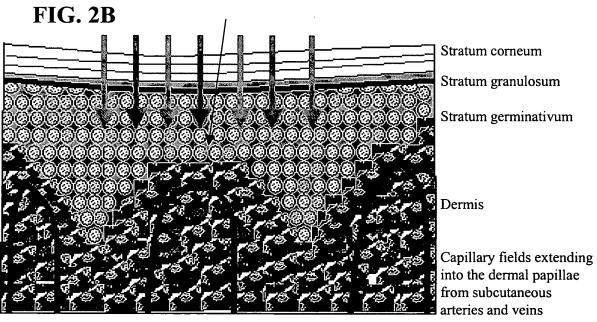
FIG. 1

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FIG. 2A

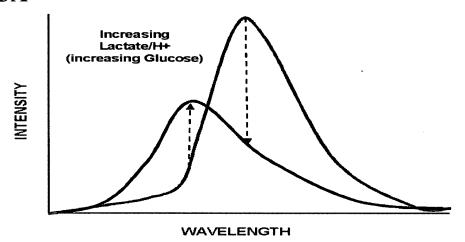


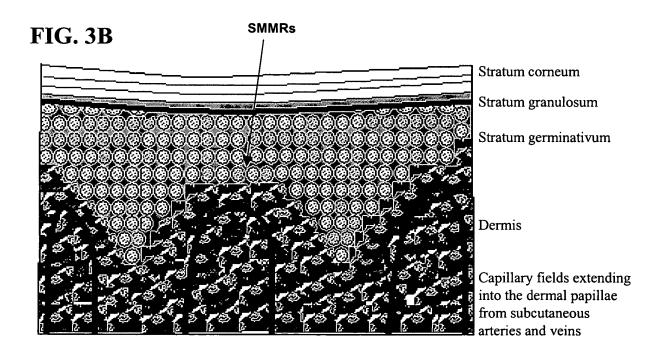


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FIG. 3A





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FIG. 4A

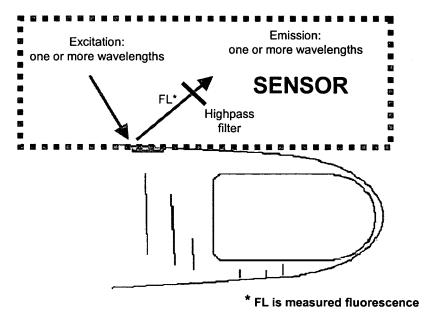
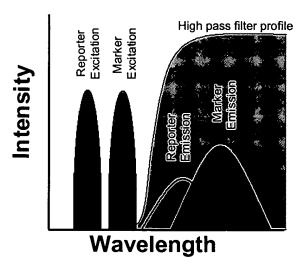


FIG. 4B



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FIG. 5A

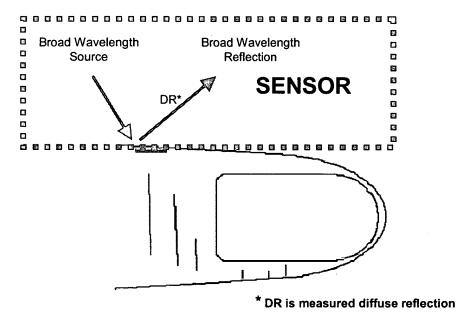
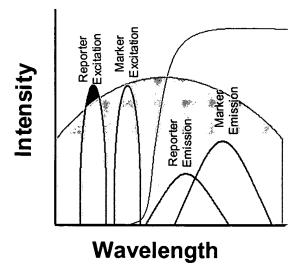


FIG. 5B



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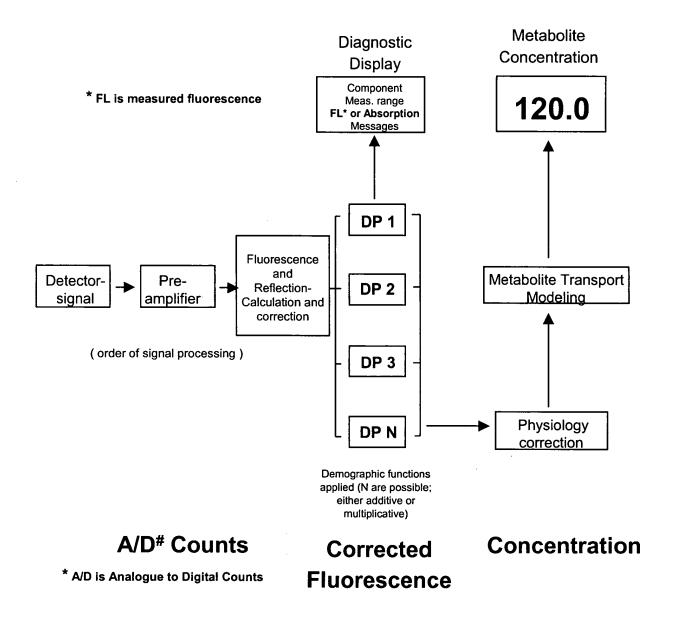


FIG. 6

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Inventors: Workman, Lambert and Coleman
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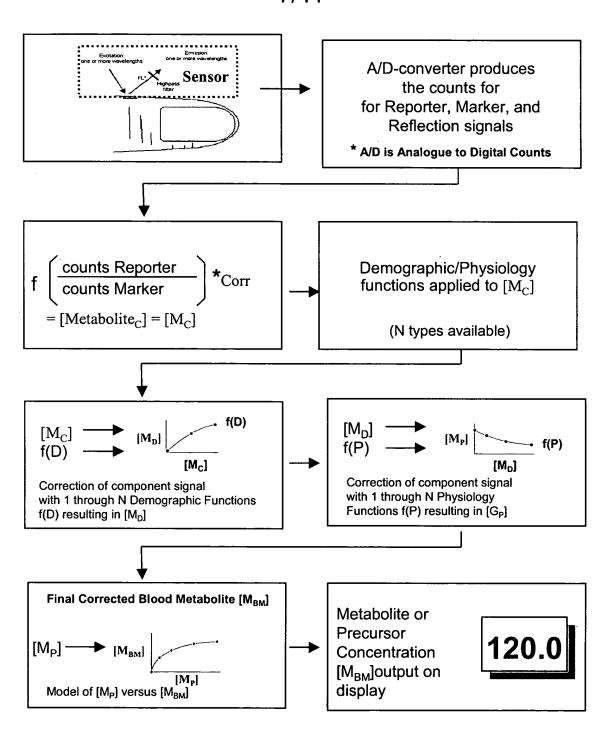


FIG. 7

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Inventors: Workman, Lambert and Coleman
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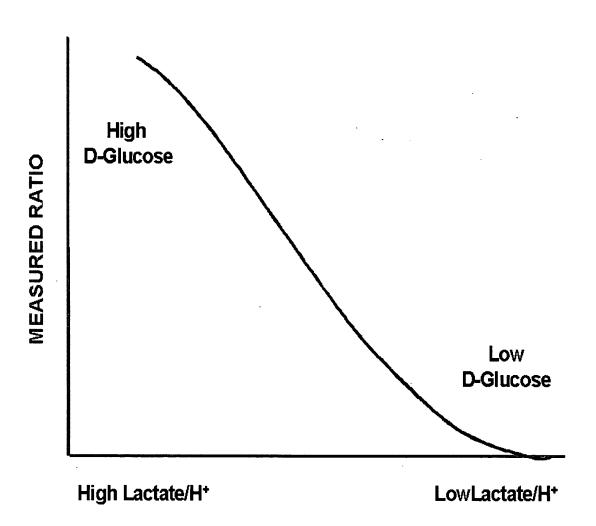


FIG. 8

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FIG. 9A

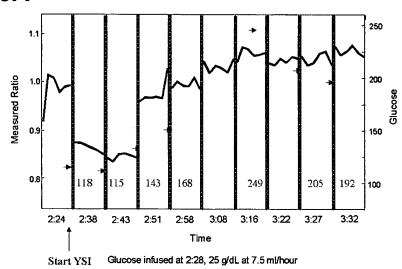


FIG. 9B

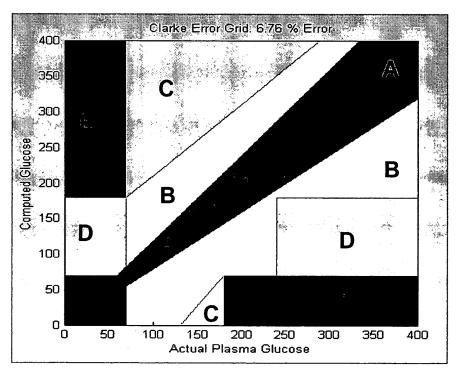
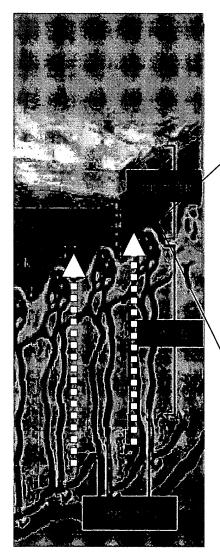
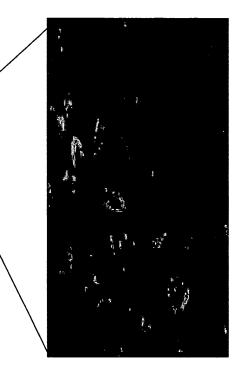


FIG. 10A

FIG. 10B



Human skin

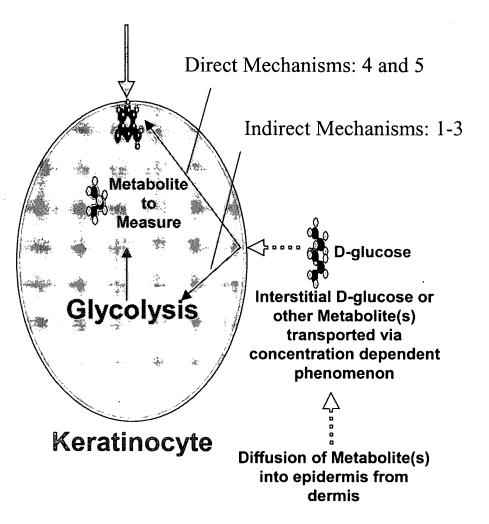


Human keratinocytes

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Disposable patch added to skin surface - passive transport of SMMR to keratinocytes

SMMR



asive measurement of analytes rkman, Lambert and Coleman Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000

FIG. 11

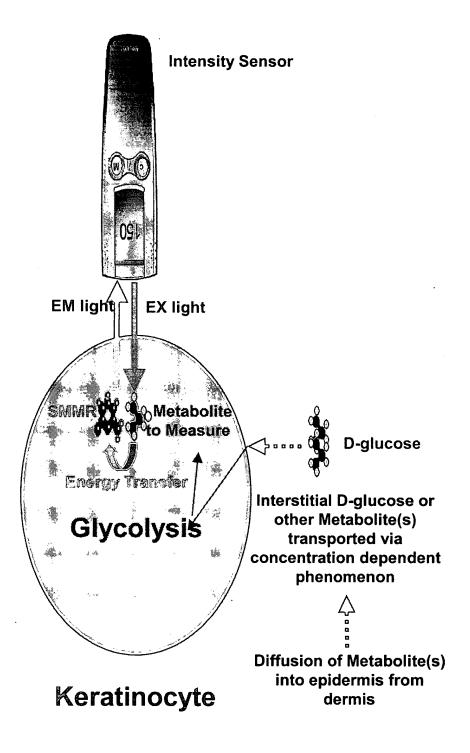


FIG. 12

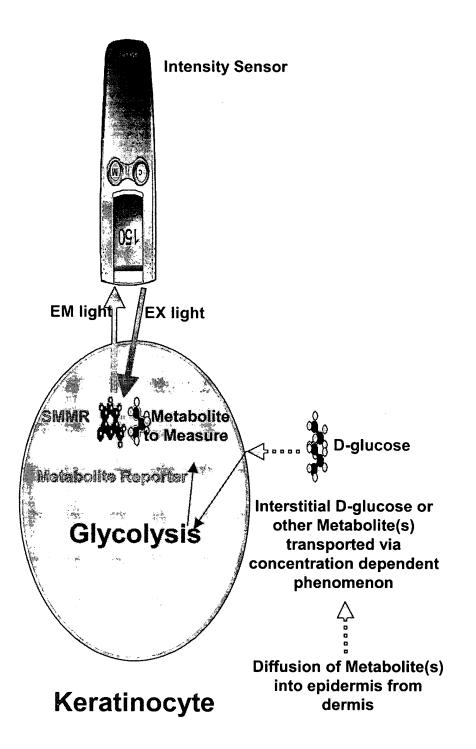


FIG. 13

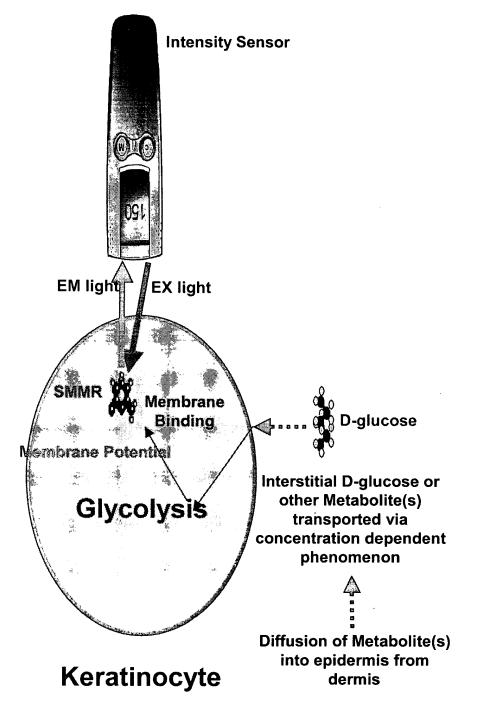


FIG. 14



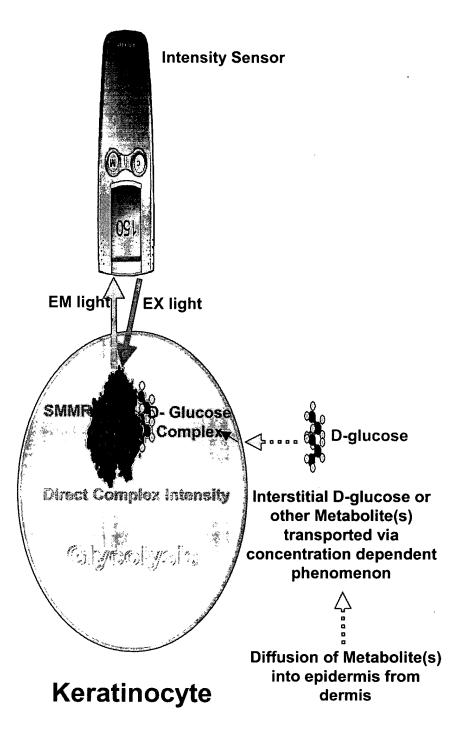


FIG. 15

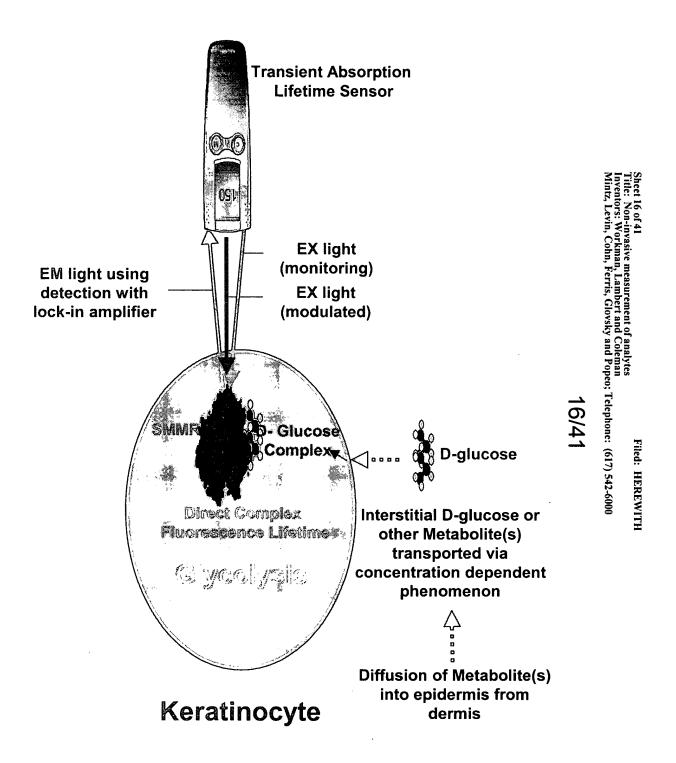


FIG. 16

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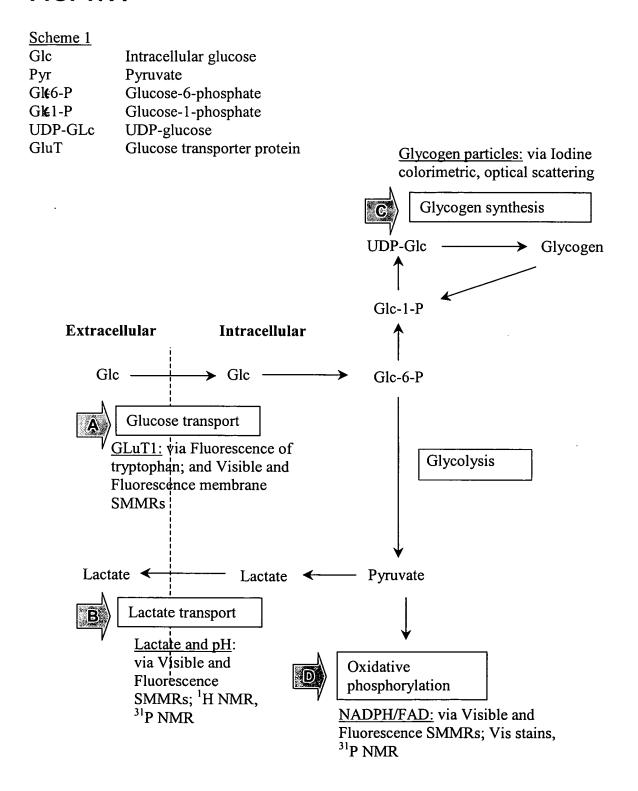
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FIG. 17A



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FIG. 17B

Scheme 2. Overview of metabolic pathways for glucose in epidermis

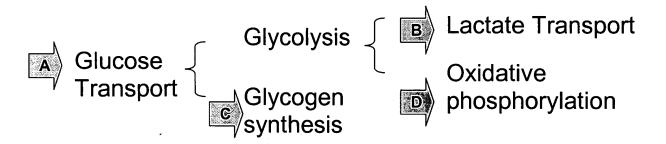


FIG. 17C

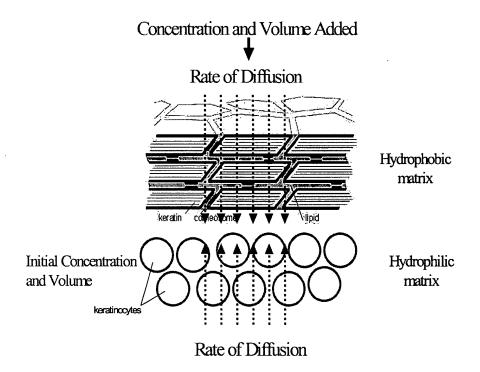
Scheme 3. Structure of generic pH sensitive dye for specific action as a lactate/H⁺ SMMR

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Scheme 4. In Vivo Calibration Issues

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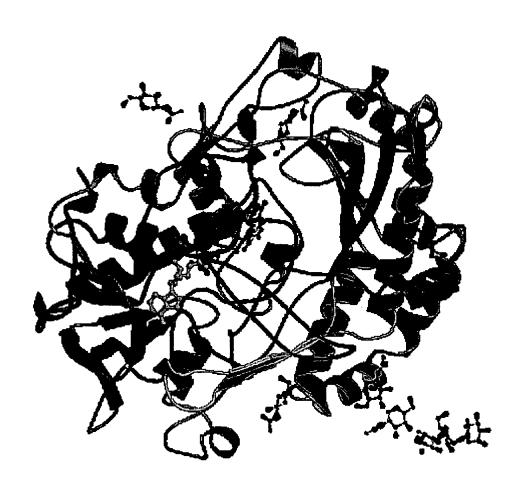


FIG. 18

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Inventors: Workman, Lambert and Coleman

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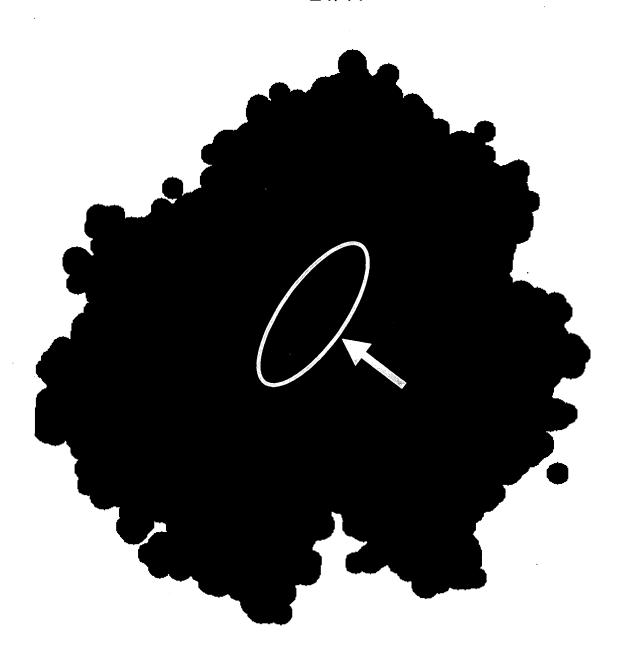


FIG. 19

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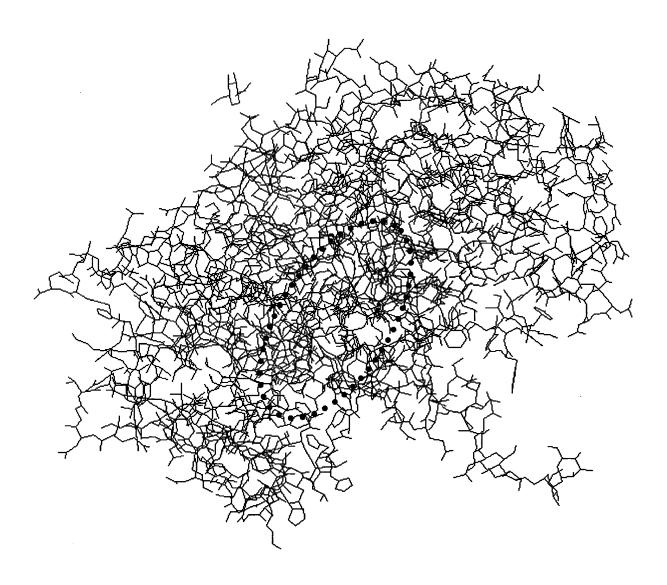


FIG. 20

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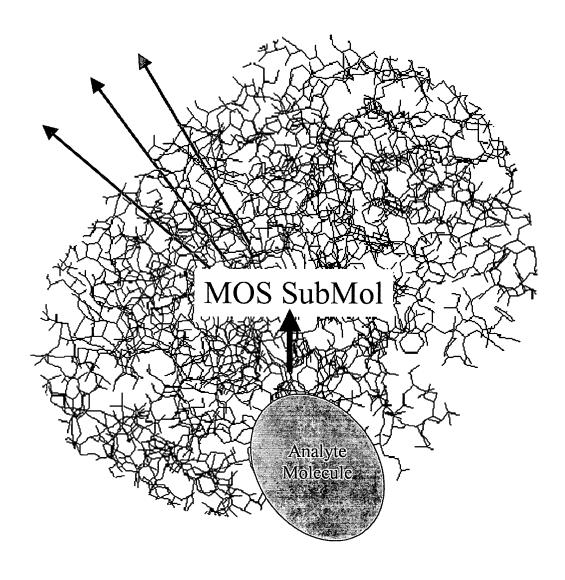
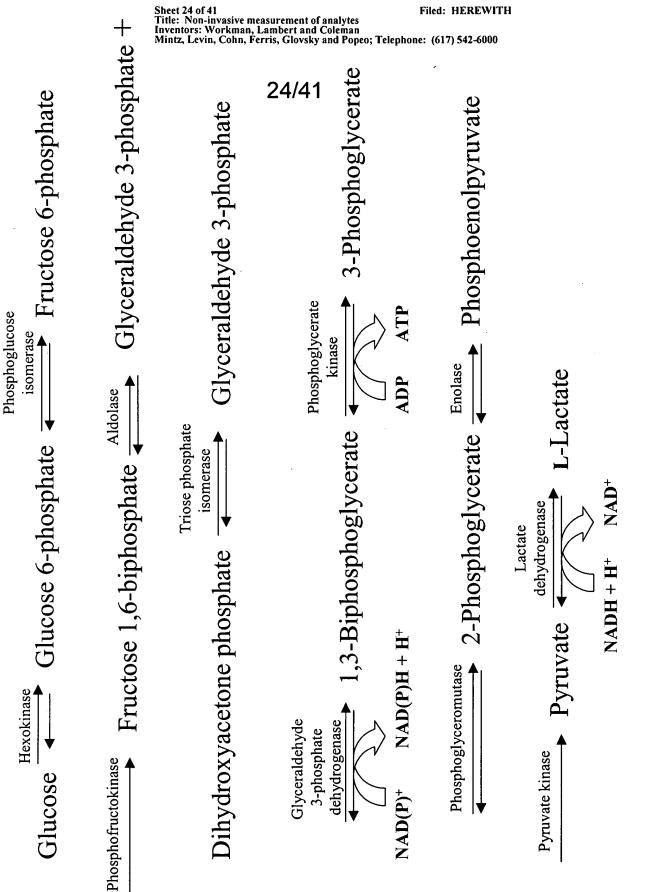


FIG. 21



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Title: Non-invasive measurement of analytes
Inventors: Workman, Lambert and Coleman
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000 Glyceraldehyde 3-phosphate 25/41 3-Phosphoglycerate Fructose 6-phosphate Phosphoenolpyruvate Glyceraldehyde 3-phosphate ATP^* Phosphoglycerate kinase Phosphoglucose somerase Enolase ADP Aldolase Triose phosphate 1,3-Biphosphoglycerate 2-Phosphoglycerate Glucose 6-phosphate somerase Fructose 1,6-biphosphate NAD⁺ dehydrogenase Lactate Dihydroxyacetone phosphate NADH* + H⁺ Phosphoglyceromutase Hexokinase dehydrogenase Glyceraldehyde 3-phosphate Glucose* Pyruvate kinase Phosphofructokinase NAD(P)+

*Detectable Analytes (direct or indirect)

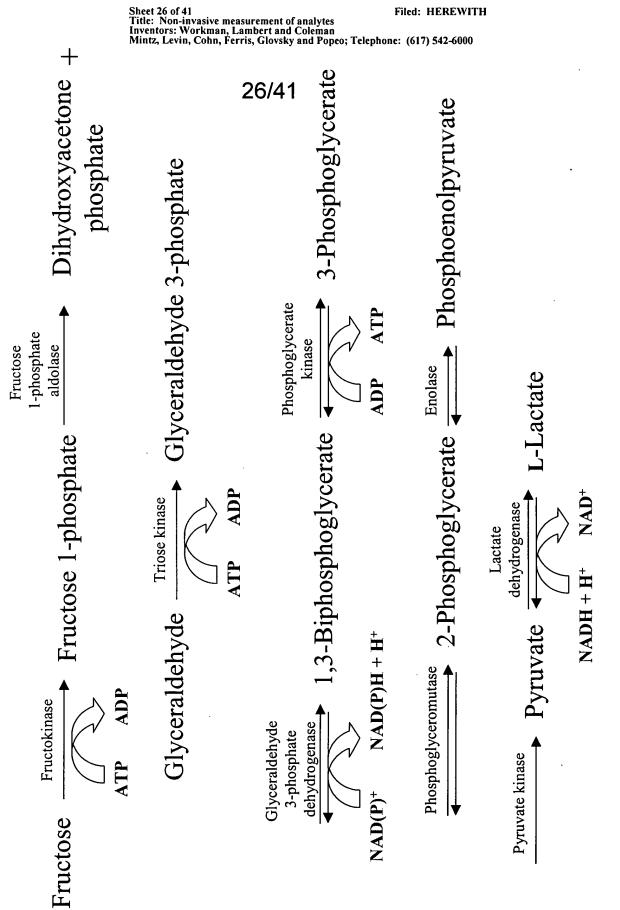
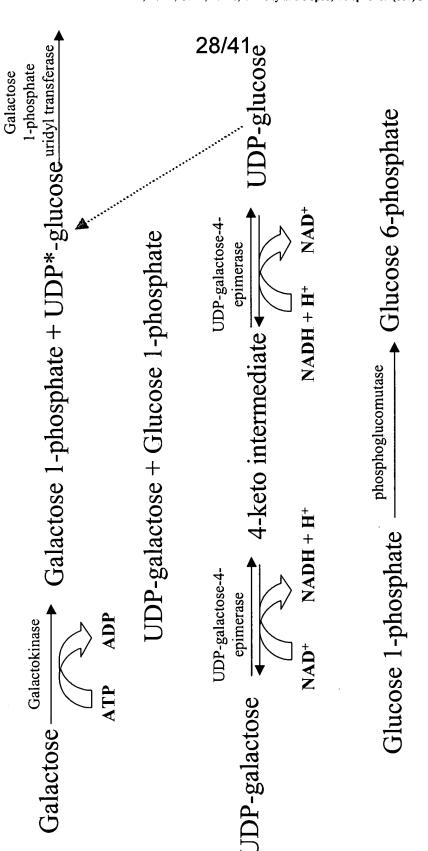


FIG. 24

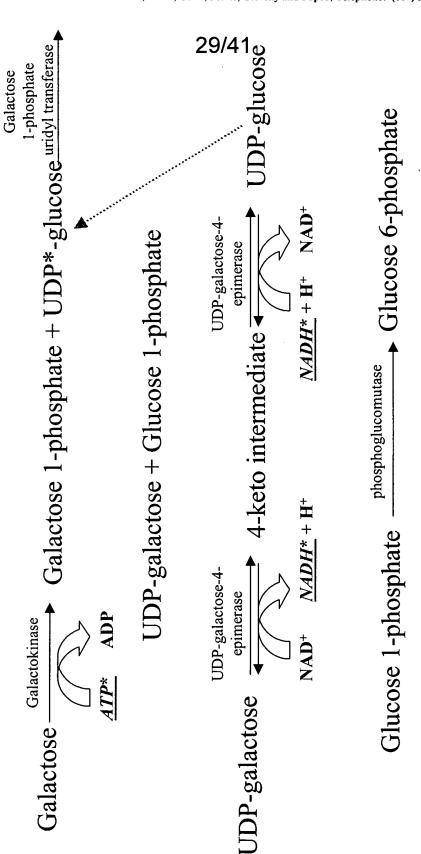
*Detectable Analytes (direct or indirect) FIG. 25

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Continuation of Glycolysis

*uridine diphosphate (UDP)



Continuation of Glycolysis

*uridine diphosphate (UDP)

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SMMR Mechanisms of Signal

1.0 Enhancement of Signal-to-noise of native autofluorescence

(boosts signal by 5 to 50) indicating redox transfer coenzyme activity 1.1 Energy Transfer from NADH, NAD(P)H, or FAD to Reporters within cells and tissues

1.2 Redox potential Reporters indicates number of mitochondrial transmembrane redox potential events

2.0 Enhancement of Specific Metabolite and Precursor Signals

2.1 Lactate Reporters indicate lactate formation from anaerobic glycolysis

2.2 Ca²⁺ Reporters indicate available ATP and ion pump transport activity fueled by glycolytic activity

3.0 Direct Glucose Reporters indicating quantitative levels of d-glucose

3.1 Protein-labeled fluorophores

3.2 proteins with a photooxidizable cofactor (such as FAD) to observe

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Mintz, Levin, Cohn, Ferris, Glovsky and Popeo; Telephone: (617) 542-6000

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Glucose + 2 P_i + 2ADP 2NAD $^+$ \rightarrow 2Pyruvate + 2 NADH + 2 ATP + 2H $^+$ + 2H $_2$ O

NADH Energy

Transfer

Glycolysis within the cell Cytosol (A)

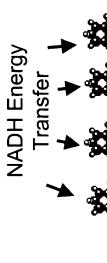
Energy Transfer Reporters

Small Molecule Metabolic Reporters (SMMR)

Cell Cross-section

Within the Mitochondria (B)

NAD⁺ + Pyruvate + CoA → Acetyl CoÀ + CO₂ + NADH



Small Molecule Metabolic Reporters (SMMR)

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NAD⁺ + Pyruvate + CoA → Acetyl CoA + CO₂ + NADH + H⁺

In the Mitochondria

Redox Potential Reporters

reporter (SMMR) units to attach to the membrane. This causes Increase in glucose concentration increases the mitochondrial membrane potential causing more small molecule metabolic fluorescence quenching proportional to changes in glucose concentration



Inner mitochondrial membrane with bound SMMRs



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Glucose + 2P_i +2ADP → 2 lactate +2ATP + 2H₂O

Anaerobic Glycolysis

Lactate Reporters

33/41 Increase in glucose concentration increases the lactate

concentration. The pH changes are directly related to glucose formation in a 2:1 ratio. A small molecule metabolic reporter (SMMR) is used to detect pH changes caused by lactate

pH change reduces FL of SMMRs

concentration



Ca²⁺ Reporters Ca²⁺ and ATPase

34/41 cell performs a signaling action Ca^{2^+} is released from ion storage into After signaling the Ca2+ is pumped back into storage using ATPase \mathbb{C} a²⁺. If the ion pumps are not working due to respiratory stress the metabolic reporter (SMMR) is used to detect Ca^{2+} changes caused Cell signaling is accomplished using ions such as Ca2+. When the concentration changes are directly related to healthy cell function. synthesized from Available ATP. Each molecule of ATP pumps 2 incapacitated. The ion concentration gradients are maintained by the cytosol where it triggers cellular activities. A small molecule ion concentrations equilibrate by diffusion since the pumps are by changes in ion concentration within the cytosol. The ${\sf Ca}^{2+}$ ATP regulated pumps.

Ca²⁺ changes increase FL of SMMRs



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Glucose + $6O_2$ +36 ADP +36P_i \rightarrow 6CO₂ + 6H₂O +36 ATP + Heat

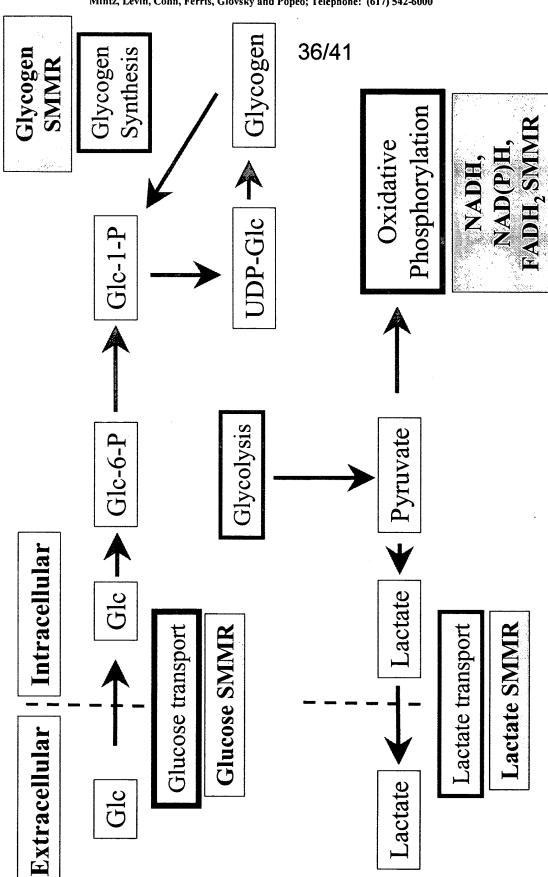
Aerobic Respiration

O₂ Reporters

environment for aerobic respiration. A small molecule metabolic environment. The O₂ changes are directly related to ability to reporter (SMMR) is used to detect O_2 changes in the cellular Increase in molecular oxygen indicates a favorable manufacture ATP.

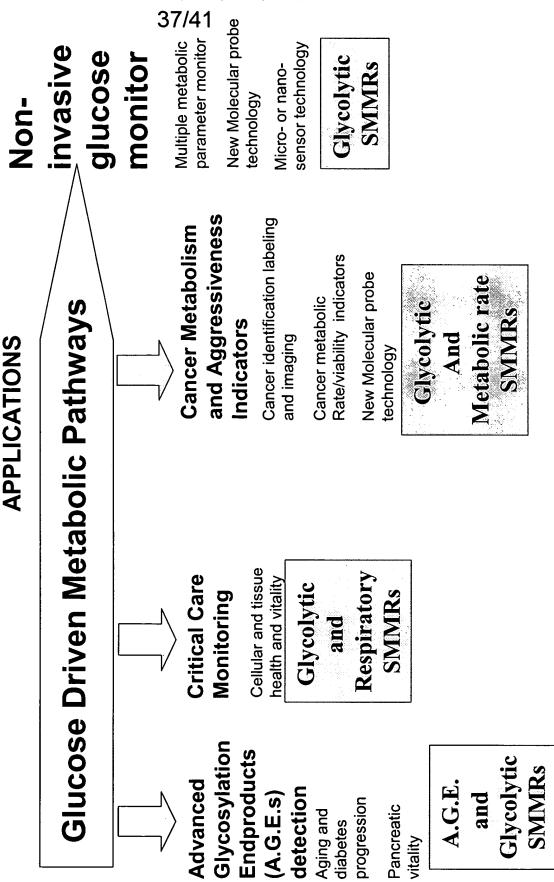
O₂ changes increase FL of SMMRs





SMMR has been used to establish analytical methods for measuring each glucose pathway for a variety of cell types

SMMR Application Summary



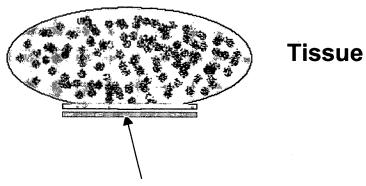
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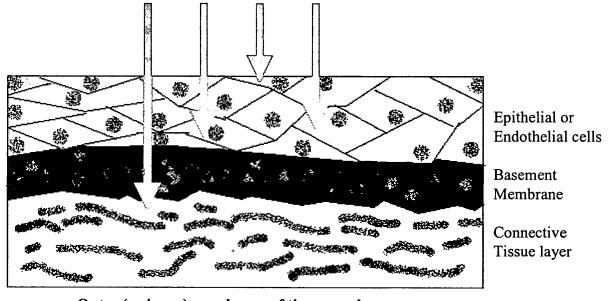
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Method for adding SMMR to peripheral epithelial cells in tissues and organs



A. SMMRs are applied to tissue surface

B. SMMRs are transported for up to 10-300 microns into the top of the tissue using passive or active transport



Outer (or inner) membrane of tissues and organs

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39/41 SMMR for Metabolite Discrimination or Imaging

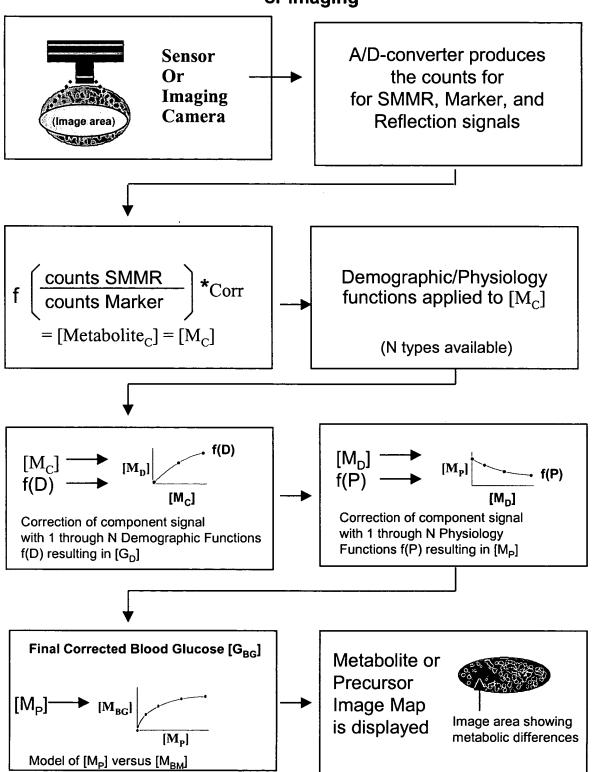


FIG. 37

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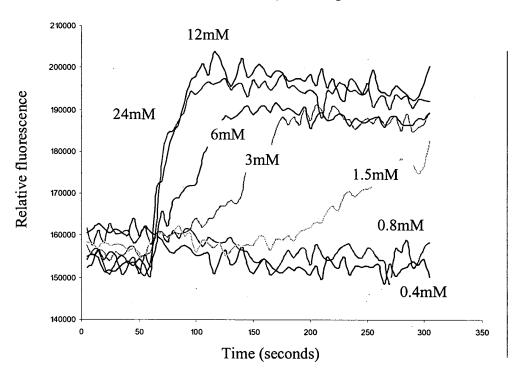
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CW Experiments - Fluorescent Response vs Glucose Addition (Concs in mmol) - averaged data



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Plot of phase shift vs transient lifetime with a modulation frequency of 2 x 10 Hz

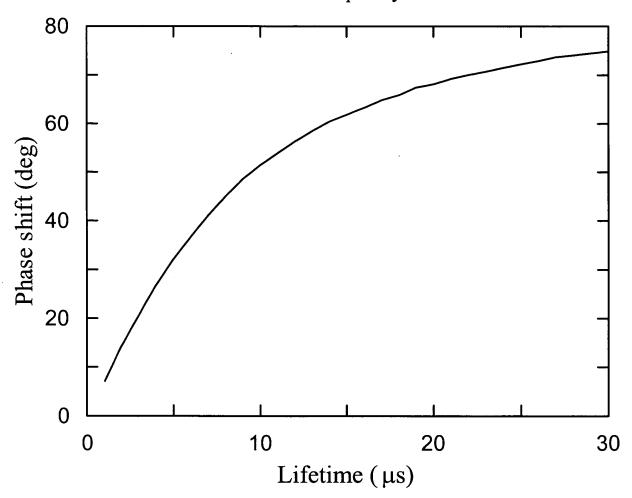


FIG. 39